

## 2022 Research Day Abstracts

Abstracts are listed in alphabetical order by the presenters' last names.

### **Viral Trojan horse: Exosome formation mediates BVDV transmission to susceptible cells**

**Dr. Morgan Adkins, Dr. Andi Lear, Dr. Marc Caldwell, Dr. Jon Beever, and Dr. Mohammed Abouelkhair**

The primary objective of this study was to evaluate the transmission of bovine viral diarrhea virus (BVDV) to susceptible cells via exosome-mediated transmission. BVDV is an endemic pathogen of North American cattle herds with the ability to infect other Artiodactyla species and cause a variety of clinical outcomes including immunosuppression, bovine respiratory disease complex, abortion, and fetal infection. Historically, direct cell to cell has been defined as the main mechanism of BVDV infection within a host, however the aim of this study was to demonstrate a novel route of cellular infection. Madin-Darby bovine kidney cells (MDBK) were propagated in sterile cell culture and inoculated with a cytopathic and non-cytopathic strain of BVDV, Singer and New York-1, respectively. Following inoculation, infection of cells was confirmed with RT-qPCR. The cell culture media was collected, and exosomes were captured and purified via CD63 Exo-Flow capture kit using magnetic streptavidin beads. During purification, exosomes were washed with anti-BVDV antibody to bind free virus particles then exosome collection was confirmed via flow cytometry. Naive MDBK cells were inoculated with exosomes from the three treatment groups: Singer strain, NY-1 strain, and a naive control. Viral infection to the cells via exosome transmission was then confirmed using RT-qPCR. Based on these findings, this experiment demonstrates in-vitro exosome mediated BVDV infection to naive MDBK cells and thus provides evidence of a novel route of BVDV transmission.

### **Novel arthropod exosomal cement protein mediates transmission of tick-borne flavivirus by modulation of human skin chemokines**

**Dr. Waqas Ahmed, Dr. Girish Neelakanta, and Dr. Hameeda Sultana**

Ixodidae ticks secrete a substance called cement that supports in anchorage of tick mouthparts to the host skin for longer feeding, to cement/seal the feeding cone/cavity for directional blood flow and to defend from being groomed off by the vertebrate host. The molecular mechanisms for secretion of these cement proteins at the feeding pit are not understood. We hypothesize that tick exosomes may facilitate cement secretion and mediate their important functions during feeding. Therefore, we combed the *Ixodes scapularis* genome and tested ten different cement molecules for their expression during feeding, role in pathogen transmission and being present in exosomes. Only one upregulated tick cement molecule (both *in vitro* and *in vivo*) was detected in tick exosomes. Our previous studies have shown that exosomes, play crucial role in facilitating the transmission of vector-borne flaviviruses. The current study provides evidence for the presence of full-length RNA genome of Langat virus (LGTV), a tick-borne flavivirus similar to tick-borne encephalitis virus. We addressed the role of novel exosomal cement function in pathogen transmission and in modulations at the tick-host skin interface. Our data showed that RNAi mediated silencing of this exosomal cement resulted in reduced LGTV loads in all tested samples (during both acquisition or transmission of pathogen). Silencing of tick cement molecule distinctly reduced feeding efficiency, tick body weights and LGTV transmission to the mammalian host. Our previous study revealed that treatment of human-skin keratinocytes with tick saliva/salivary gland or ISE6 tick cell-derived exosomes upregulates human chemokines such as interleukin-8 (IL-8) and downregulates C-X-C motif chemokine ligand 12 (CXCL-12) to delay cell migration, wound healing and tissue repair. We now found that exosomal cement facilitates these modulations. Collectively, we have identified and characterized the role for novel tick exosomal cement protein that facilitates blood feeding and transmission of pathogens by modulation of human skin chemokines. This is a first transformative study that opens new line of investigation to target novel exosomal cement protein in transmission of tick-borne flaviviruses.

### Characterizing *Mycobacterium* isolates from dogs and cats

Jaclyn Azelby, Porsha Reed, Rebekah Jones, Brian Johnson, and Dr. Sree Rajeev

Infections caused by nontuberculous *Mycobacterium spp.* have been an increasing cause of morbidity in humans and animals, ranging from localized granulomatous infections of the skin and subcutis to disseminated disease. These infections are often challenging to diagnose and treat due to the diversity of species causing these infections, and hence the variation in antimicrobial susceptibility patterns. In this study, we characterized mycobacterial isolates obtained from dogs and cats representing both in house and referral cases submitted between 2016 and 2022 at the University of Tennessee Veterinary Medical Center. We performed a variety of tests, including Gram staining, Acid-fast staining, Matrix-Assisted Laser Desorption/Ionization Time-of-flight mass spectrometry (MALDI-TOF MS), 16s rRNA amplification and sequencing with two different sets of primers for the identification of these isolates and antimicrobial susceptibility testing on all the isolates. We observed a discrepancy in identifications made by MALDI-TOF and those made by DNA sequencing with the two different sets of primers. Of the 25 samples with an identification by both MALDI-TOF and sequencing using the primer pair-1, only nine samples (36%) had a correct match. Of the 24 samples with an identification by both MALDI-TOF and sequencing using primer pair 2, 15 samples (62.5%) had a correct match. Antimicrobial susceptibility testing by broth microdilution test identified variations in patterns between isolates. Characterization of genotypic and phenotypic attributes of mycobacterial isolates in combination with their antimicrobial susceptibility pattern could serve as an important tool for improved diagnostic care and more effective clinical treatment.

### Prevalence and histologic lesions of *Trypanosoma cruzi* in Tennessee wildlife

Dr. Eliza Baker and Dr. Rick Gerhold

*Trypanosoma cruzi* is a vector-borne protozoan parasite that affects an estimated 8 million people. Though uncommon in the United States, cases of locally acquired infections have expanded dramatically in recent years, leading to concern that *T. cruzi* is an emerging disease in the United States. Numerous wild mammal species are known to carry the parasite in the southeast, with raccoons and opossums some of the most frequently infected. Surveys in Tennessee are lacking, despite the presence of the vector in the area. We sampled 88 raccoons, fifteen Virginia opossums, four skunks, and three foxes for the presence of *T. cruzi* DNA in heart and blood. 13% of raccoons were positive for *T. cruzi*, while none of the other mesocarnivores tested positive. In the samples with histopathology available (n=48), myocarditis was significantly more likely to occur in positive cases than negative cases. PCR of the heart was more sensitive than PCR of blood, and the majority of the positive cases occurred in the fall. This survey sheds light on the prevalence and potential health impacts of *T. cruzi* in local wildlife. Due to the high prevalence of this parasite in the area, research into the prevalence of *T. cruzi* in domestic dogs, particularly those with myocarditis or heart failure, is warranted.

### Functional and structural characterization of potential immunogenic proteins of pathogenic *Leptospira*

Dr. Liana Nunes Barbosa, Dr. Alejandro Llanes, Myranda Gorman, and Dr. Sree Rajeev,

We applied reverse vaccinology strategy using advanced bioinformatic, immunoinformatic and proteomic analyses to identify three immunogenic *Leptospira* proteins, predict their 3-D structures, functions, and immunogenic potential as vaccine candidates to prevent *Leptospira* infection. Three-dimensional (3-D) structural modeling was predicted on I-TASSER and Protein Data Bank (PDB). Plasmids containing the target genes were purchased commercially and inserted into competent cells of *E. coli* BL21 (DE3) by heat shock. Western blots were performed following an SDS-PAGE run and the reactions antigen-antibody were read using chemiluminescent substrate in a Blot Scanner. Protein 1 predictions suggest this protein presents an Ycel-like protein domain, which commonly contain 8-stranded  $\beta$ -barrel fold and can play an important function in the metabolism and/or transport of compounds. Protein 2 was predicted as possibly an integral transmembrane protein containing an  $\alpha$ -helical region, which is common to chemoreceptors and histidine kinases. It is suggested this protein can be part of a family of bacterial receptors that mediate chemotaxis to diverse signals, presenting both methyl-accepting chemotaxis protein and HAMP domains. The protein 3 was predicted as a hypothetical protein containing a transmembrane  $\alpha$ -helix and a non-cytoplasmatic domain/regions. The protein 2 and 3 were expressed in their insoluble forms, presenting the expected molecular masses of 22.3 kDa and 24.5 kDa, respectively. Our results suggest that two proteins identified and expressed are integral membrane proteins that in theory are easily accessible to host immune system components, and can be potential candidates to develop a vaccine to prevent *Leptospira* infection.

### Bone cement: Factors affecting exothermic polymerization

Kendall Barnes, Dr. Kristin Bowers, Dr. David Anderson, Lori Terrones, and Elizabeth Croy

Polymethylmethacrylate (PMMA), a.k.a. bone cement, hardens by an exothermic polymerization reaction. The goal of this in vitro study was to characterize thermal changes that occur during polymerization of bone cement under various conditions. The main clinical concern was bone thermal necrosis, as the bone thermal limit is  $-50^{\circ}\text{C}$  for  $>1$  min (Kniha, Heussen et al. 2020). The objectives, to determine the effect of various factors on exothermic characteristics, were: volume of bone cement used, type of bone cement used, implant presence within a cement column, and a lavage cooling system (room temperature vs chilled). Plastic syringe casings were used as the molds. A human bone cement product, Simplex P, and two veterinary products, EUROFIX GUN (low viscosity) and EUROFIX RO, were used in the different objectives. Cannulated stainless steel and titanium screw were used as the metallic implants. The most significant factors affecting the maximum temperature and duration above  $50^{\circ}\text{C}$  were cement volumes, products, and cooling lavage systems. The presence of a metallic implant had no effect on temperature or time.

### Dengue virus modulates cell cycle signaling in human megakaryocytes

Swarnendu Basak, Dr. Girish Neelakanta, and Dr. Hameeda Sultana

In the recent years, 50-100 million dengue infections have been reported annually, with 500,000 hospitalizations and 22,000 deaths, thus suggesting dengue as a global problem, worldwide. Currently, there are no effective treatments or vaccines available for dengue infection. To understand dengue virus (DENV) caused thrombocytopenia, we studied the cell cycle in megakaryocytes, that were primarily responsible for the production of platelets, which helps in blood coagulation in our body. Suppression of megakaryocytes by DENV infection significantly reduces the number of platelets count or other hematopoietic cells that eventually leads to dengue hemorrhagic fever (DHF), followed by dengue shock syndrome (DSS) and death. Although, megakaryocytes are known for its complex endomitotic cell cycle (with partial mitosis, the cells enter mitosis and progress through normal prophase, pro-metaphase, metaphase, and up to anaphase A, but not to anaphase B, telophase, or cytokinesis), however, it is not clearly understood how DENV uses these cells for its replication. Therefore, understanding the modulation of DENV-mediated cell regulation of human megakaryocytes at the intracellular level is highly important. Using a leukemic *in vitro* megakaryocytic cell line, MEG-O1 cells, derived from bone marrow of a leukemic patient, we show that DENV infection significantly modulates the cell cycle molecules. Protein profile microarray data analysis showed significant up regulation of several of the cell cycle regulatory proteins including CDK4, Cyclin B1, and CDK1 and down regulation of Chk1, Cyclin D3 and E2F-3. Quantitative real time PCR and immunoblotting analyses, further confirmed up regulations of CDK4, Cyclin B1, and CDK1 genes in presence of DENV infection. In addition, lower amounts of Aurora B Kinase expression suggested that DENV allows the Meg-O1 cells to enter into anaphase stage. Interestingly, immunoprecipitation analysis revealed a strong interaction between cyclin B1 and CDK1 that perhaps enhances Meg-O1 cells to enter and complete anaphase during DENV infection. Overall, our study suggests that DENV may likely correct the cell cycle process in megakaryocytes by interfering with cell cycle regulatory proteins that eventually, leads to moving the mitosis process. In conclusion, we are reporting, a very important molecular insight regarding the DENV-mediated cell cycle modulation in human megakaryocytes.

### Pharmacokinetics and pharmacodynamics of pantoprazole in sheep

Kailee Bennett, Dr. Joe Smith, Dr. Pierre-Yves Mulon, Jessica Gebert, Joan Bergman, Dr. Jessica Garcia, Olivia Escher, Lainey Harvill, Ryan Flynn, and Dr. Sherry Cox

Sheep suffer from abomasal ulcers caused by factors like diet, non-steroidal anti-inflammatory drug (NSAID) treatment, and bacterial infections with few options for treatment. Pantoprazole is a proton pump inhibitor (PPI) used for gastric ulcers, gastritis, and esophagitis in humans. Pantoprazole offers a parenteral method of administration in species, like sheep, where oral PPIs are ineffective. Four adult ewes underwent abomasal cannulation and were administered a 1 mg/kg intravenous (IV) or 1 mg/kg subcutaneous (SQ) dose of pantoprazole for three consecutive days. Blood samples were collected from the ewes at timed intervals over 24 hours, and abomasal fluid samples were collected over 96 hours. Plasma concentrations were analyzed via reversed-phase liquid chromatography. The drug was eliminated from the system rapidly, with a 4.75 hour half-life after IV administration and a 3.96 hour half-life after SQ administration. Both routes of administration had similar values for total exposure [Area under the Curve: 17,766 hr\*ng/mL for IV dose, 14,961 hr\*ng/mL for SQ], with a SQ bioavailability of 88%. After drug administration, the abomasal fluid pH was significantly ( $P < 0.05$ ) higher than pre-pantoprazole pH levels up to 8 hours after dosing on all three days for both treatments. This investigation indicates that pantoprazole offers a potential solution to abomasal ulceration in sheep by raising the gastric fluid pH. Similarities in IV and SQ administration parameters suggests that SQ administration is efficacious, and could make dosing easier for practitioners and clients. Further studies are needed to determine adverse effects and withdrawal times.

### Mesenchymal stem cell use in acute tendon injury: In vitro tenogenic potential vs. in vivo dose response

**Dr. Kristin Bowers**, Dr. Lisa Amelse, Dr. Austin Bow, Dr. Steven Newby, Dr. Amber MacDonald, Dr. Xiaocun Sun, Dr. David Anderson, and Dr. Madhu Dhar

Stem cell therapy for the treatment of tendon injury is an emerging clinical practice, but questions persist regarding the overall tenogenic potential and efficacy of this treatment alone. In this study, we aimed to assess the effects of growth factor exposure in vitro on rat mesenchymal stem cell morphology, behavior, and tendon-associated glycoprotein production, and we aimed to assess the therapeutic potential of intralesional stem cells, as a function of dose, in vivo. First, rat adipose-derived (rAdMSC) and bone marrow-derived (rBMSC) stem cell lineages were isolated, characterized, and compared in terms of proliferation and cellular viability. Next, the tenogenic differentiation potential of the rAdMSC lineage was tested through stimulation with reported tenogenic growth factors, transforming growth factor (TGF)- $\beta$ 3 and connective tissue growth factor (CTGF). The most effective tenogenic factor in terms of cellular morphologic change, cell alignment/orientation, sustained cellular viability, and tendon-associated glycoprotein upregulation was TGF $\beta$ 3. Finally, the therapeutic potential of undifferentiated rAdMSC as a function of dose was assessed using a rat acute Achilles tendon injury model. On gross morphology, rAdMSC-treated tendons exhibited fewer adhesions and scar tissue than control tendons. However, regardless of rAdMSC dose, no significant differences in histological grade or tissue collagen I deposition were noted between rAdMSC-treated and control tendons. Collectively, rAdMSC exhibited appropriate stem cell markers and tenogenic potential in vitro, but clinical efficacy of intralesional implantation of undifferentiated cells in acute tendonitis cases could not be proven. Further investigation into complementary therapeutics or specialized culture conditions prior to implantation are warranted.

### Comparison of the Truforma cortisol assay and a chemiluminescent assay for measurement of canine serum cortisol concentrations

**Cody Brady**, Dr. Shelly Olin, Dr. Luca Giori, and Dr. Jacqueline Whittemore

Management of hyperadrenocorticism and hypoadrenocorticism in dogs requires both the accurate recognition of clinical signs and confidence in the laboratory test used for diagnosis. Currently, reference laboratories are used for confirmation of ACTH stimulation and low-dose dexamethasone suppression tests, which not only increases the cost of diagnosis, but also the time required for accurate diagnosis. Truforma is a novel, non-fluorescent assay (Zomedica, Ann Arbor, MI) that allows for rapid, in-house cortisol measurement. Internal assay validation has been performed, but there are no peer-reviewed studies to affirm its clinical use. The purpose of this study is to determine the precision of the Truforma cortisol assay and assess agreement between it and a chemiluminescence assay (CLA; Immunlite 2000 XPI, Siemens Healthcare Diagnostics Products Ltd, Los Angeles, CA) for measurement of serum cortisol concentration in dogs. We hypothesize that the Truforma cortisol assay will have good precision and agreement with CLA at low, medium and high cortisol concentrations with minimal bias or impact on clinical decision making. Frozen serum samples from the Diagnostic Endocrinology Service at UTCVM collected between March and July of 2022 were selected based on original cortisol concentration (0.5-30 ug/dL). To assess precision of the Truforma assay, three pools of low, medium, and high cortisol concentrations were made to be run in triplicate over five consecutive days. For the method comparison, n=60 samples were aliquoted for testing CLA and Truforma in duplicate. Data analysis will include descriptive statistics, Passing-Bablok regression analysis, and Bland-Altman difference plot analysis. Data collection is ongoing.

### **A synthetic kisspeptin analog, compound 6, increases plasma luteinizing hormone in cows with low plasma progesterone concentrations**

**Kara Brady**, Allison Renwick, Dr. Rebecca Payton, Dr. Lannett Edwards, Dr. Casey Nestor, Dr. Massimiliano Beltramo, and Dr. Brian Whitlock

Kisspeptin (KP) is a neuropeptide that is an integral part of the hypothalamo-pituitary-gonadal (HPG) axis and reproductive physiology. KP is responsible for stimulating gonadotropin releasing hormone (GnRH) and subsequently luteinizing hormone (LH) secretion. Compound 6 (C6), a synthetic kisspeptin analog, may have greater therapeutic effects than KP due to higher potency and longer half-life. The objective of this study was to evaluate the effect of C6 on plasma LH concentrations in cows with a low plasma progesterone concentration. Six cows were administered one of three treatments in a crossover experiment (one replication; about one month between treatments; n=4/treatment) – control (2 mL saline), KP (100 nmoles), or C6 (100 nmoles). Additionally, intravaginal progesterone-releasing devices were used in all cows so that differences in endogenous hormone profiles would not confound results. Treatments were administered intramuscularly on day 6 of follicular wave emergence and blood samples were collected every 12 minutes for 7 hours (1 hour pre- to 6 hours post-treatment) and then hourly for 6 additional hours for analysis of plasma LH concentration. Upon preliminary evaluation, C6 appears to have increased both LH pulse amplitude and serum LH concentrations when compared to control and KP groups. Complete statistical analysis is pending.

### **Parasites of wild turkeys in Middle Tennessee**

**Katelyn Broadway**, Laura Horton, Dr. Rick Gerhold, Roger Shields, Lindsey Phillips

Wild turkeys play an important role in hunting as both a food source and in ecosystem conservation efforts; however, their populations have seen significant declines in North America. Recent data has suggested declines in Middle Tennessee as well. This may be attributed to their popularity in hunting both for nutritional purposes and as trophies; however, other factors have been cited for these declines such as parasites, disease, habitat loss, and climate change. There is limited data currently available on the impact and prevalence of parasites in wild turkeys. Parasites known to infect wild turkeys include *Histomonas meleagridis*, *Heterakis gallinarum*, and gastrointestinal parasites such as *Eimeria* spp., and various helminths. Due to the potential for histomonosis, a cause of mortality in turkeys, *Heterakis gallinarum* is especially important to monitor in wild populations. The sale of hunting licenses for turkeys allows for the conservation of all wildlife species; therefore, obtaining sample turkey populations is essential for conservation needs. This study evaluates the parasites found in fecal samples from a wild turkey population in Middle Tennessee which has been experiencing declines. Using a fecal flotation method, parasite eggs for 400 turkey fecal samples were categorized. Results will be discussed.



### **Pharmacokinetics of orally administered single-dose ponazuril in cats**

**Dr. Catherine Burlison, Dr. Sherry Cox, Dr. Joe Smith, Dr. Jennifer Stokes, Dr. Jacqueline Whittemore, and Dr. Becky DeBolt**

Cats and kittens in animal shelters and catteries regularly suffer from severe gastrointestinal coccidiosis, which can be fatal, and there are no drugs labeled for feline coccidiosis in the United States. Ponazuril, a triazine-class drug, is increasingly used at a dose of 50 mg/kg/d, orally, for three to five days in shelter environments for coccidiosis. A single oral dose of ponazuril paste 15% (Marquis®; Merial) at 50 mg/kg was administered to six healthy adult cats. Sample analysis was completed via high performance liquid chromatography. Plasma concentrations peaked at  $7.49 \pm 2.06$  µg/ml at  $14.67 \pm 7.45$  hr post-administration. This study shows that ponazuril achieved a plasma concentration that inhibits growth of similar organisms after a single oral dose in cats. Further studies are necessary to optimize dosing for the treatment of clinical coccidiosis in cats.

### **Safety and feasibility of intraarticular ultrafiltration probes for serial synovial fluid collection in the horse: A preliminary study**

**Dr. Alexandra Carlson and Dr. Liz Collar**

Repeat sampling of synovial fluid is accomplished by needle arthrocentesis. Each joint puncture carries risk of joint contamination, infection, and damage. Ultrafiltration probes have been utilized to collect extracellular fluid from different tissues, including subcutaneous, hoof lamina, muscles, tendons, and bone. We evaluated the safety and feasibility of serial synovial fluid collection via intraarticular ultrafiltration probes as an alternative to arthrocentesis in three horses. Under standing sedation and following a nerve block, a randomly selected metacarpophalangeal joint had a customized ultrafiltration probe inserted. Arthrocentesis was performed daily on the non-probe joint and on the probe-joint at 0-, 24-, and 72-hours post-placement and following euthanasia. Synovial fluid ultrafiltrate was continuously collected into vacutainers. Limbs were evaluated for edema and effusion. Probes were maintained for 72 to 148 hours. Joint distension used on Horse #2 aided in probe placement. The probe had no issues in collecting fluid except when clots would form in the tubing or needle following standard arthrocentesis. Total solids from arthrocentesis samples was similar between probe and non-probe joints. Horses remained comfortable for the duration of probe placement. This technique may allow for more frequent fluid sampling while minimizing blood contamination of samples. While membranes detached in two of the joints, grossly, joints appeared similar between probe and non-probe joints following euthanasia. Ultrafiltration probes appear to be a feasible option for serial sampling of synovial fluid. However, further development is warranted to increase the safety of the use of these probes within the joint.

### **Cranial neurovascular anatomy of the llama (*Lama glama*) to develop an alternative surgical approach to the tympanic bullae**

**Dr. Tim Chamberlain and Dr. Robert Reed**

Tympanic bullae of South American camelids have a unique multi-compartmental internal structure resembling that of honeycomb. This unique anatomy creates challenges for refractory otitis cases requiring surgical intervention. Surgery in lesser described species can result in unanticipated complications during and after the procedure. Extensive descriptive anatomy of llama (*Lama glama*) neurovascular structures surrounding the tympanic bullae is lacking and has historically resulted in arterial hemorrhage and nerve paralysis intra- and postoperatively. Six llama heads were acquired for dissection and all major neurovascular structures were identified, described, and compared to computed tomography scans. Llama anatomy of the head remains semi-consistent with that of Old World camelids, giraffes, and alpacas; however, unique anatomic variations in the llama may be responsible for surgical complications that result in abandonment or failure. The large tympanic bullae are bordered on the caudal aspect by a large caudal auricular artery and cranially by the maxillary and retroarticular veins. Preliminary findings suggest bilateral variations of the caudal auricular, superficial temporal, and transverse facial arteries interfere with direct access to the tympanic bullae. The proposed surgical recommendations reduce challenges for clinicians attempting to address surgical otitis cases in the llama.

### **Cellular proliferation, differentiation, and morphology of pre-osteoblastic cells exposed to 2D monolayer and 3D collagen scaffold culture conditions, +/- BMP-2**

**Katherine Deal, Dr. Kristin Bowers, Dr. David Anderson, and Dr. Madhu Dhar**

Bone defects caused by injury or trauma can lead to loss of bone integrity and function creating a significant challenge for patients. Current research in the field of bone tissue engineering aims to provide alternative methods of bone remodeling and restoration without the need for grafts. Biodegradable scaffold structures have the potential to provide the appropriate environment for functional bone recovery using pre-osteoblastic stem cells as a therapeutic approach. Research on the exogenous addition of growth factors, such as bone morphogenetic protein-2 (BMP-2), has been shown to act as a recruiting molecule and aid in osteoinduction of cells. The present study explores proliferation and differentiation of MC3T3-E1 immortalized murine cells in both 2D monolayer and 3D scaffolds +/- BMP-2. Standard cell culture plates with enriched media containing 0, 10, and 50 ug/mL BMP-2 were used for MTS assay and osteogenesis trials and analyzed using phase contrast microscopy and Calcein-green stain. Collagen scaffolds, OCS-B Xenomatrix Collagen and BIOPAD equine Collagen, were tested with the addition of 50 ug/mL BMP-2. Scaffolds were submitted on Day 7, 14, and 21 for histology with alizarin red and H&E stains. Initial histological results showed cell migration towards the central area of the scaffolds and normal cell morphology, suggesting maintenance of functionality. These findings provide a proof of concept and show the need for further study in support of future bone organogenesis.



### Parasites in wild-caught *Notophthalmus viridescens* experimentally infected with *Batrachochytrium salamandrivorans*

Taylor Demers, Dr. Deb Miller, and Dr. Wesley Sheley

Amphibians are currently experiencing extinction rates over 200 times the natural extinction rate. One emerging infectious disease contributing to these declines is caused by the fungal pathogen *Batrachochytrium salamandrivorans* (*Bsal*). *Bsal* has caused mass die-offs of fire salamanders in Europe, and poses a major threat to salamander biodiversity in North America if introduced. A recent study investigated the effect that pathogen dose and environmental temperature have on *Bsal* chytridiomycosis disease progression in wild-caught *Notophthalmus viridescens* (Eastern newts; n=41). *Bsal*-associated lesions were examined histologically and *Bsal* qPCR load was measured. Incidentally, multiple types of parasites were noted histologically. Though most parasites are considered commensal to amphibians, some have detrimental effects on their hosts. Therefore, the goal of the current study was to determine if parasite load may have an effect on severity of *Bsal* chytridiomycosis infection. Parasites were classified and quantified and statistical analyses were performed to determine if a relationship exists between environmental temperature, *Bsal* exposure dose, survival time, and parasite load. The majority of individuals were found to be infected with parasites, most commonly nematodes and protozoans. Temperature was found to have a significant effect upon parasite load, with newts kept at 6°C having higher parasite loads than newts kept at 14°C. There was also a positive correlation between parasite load and survival time. However, parasitic infection does not appear to contribute to *Bsal* load. These results indicate that more research is needed to explore the relationship between parasitism and the progression of *Bsal* chytridiomycosis.

### Geographical disparities and temporal changes of COVID-19 incidence risks in North Dakota, United States

Nirmalendu Deb Nath, Md Marufuzzaman Khan, and Dr. Agricola Odoi

Background: Coronavirus disease 2019 (COVID-19) is one of the critical public health concerns due to its high infectivity and significant socioeconomic impact. However, the incidence and burden of COVID-19 differ geographically and affect some communities more than others. Therefore, the objective of this study was to investigate geographical disparities and temporal changes of COVID-19 incidence risk in North Dakota. Methods: Retrospective COVID-19 data on confirmed cases reported between March 2020 and September 2021 were obtained from North Dakota Department of Health. Monthly COVID-19 incidence risks were computed and presented as the number of cases per 100,000 persons. Spatial Empirical Bayesian (SEB) smoothed risks were computed to adjust for spatial autocorrelation and the small number of cases in some counties. Tango's flexible spatial scan statistic was used to identify both circular and irregular-shaped high-risk spatial clusters of COVID-19 incidence risks. Geographical distribution of COVID-19 incidence risks and high-risk clusters were visualized using ArcGIS. Results: County-level SEB incidence risks varied geographically and ranged from 122 to 16,443 cases per 100,000 persons. High incidence risks tended to occur in the central and south-western parts of the state, where significant high-risk spatial clusters were identified. Furthermore, the study observed two peaks (August 2020-December 2020 and August 2021-September 2021) and two non-peak periods of COVID-19 incidence risk (March 2020-July 2020 and January 2021-July 2021). Conclusion: The findings of this study are useful for guiding intervention strategies by identifying communities with high incidence risks. This information helps health professionals to better target intervention efforts.

### Antimicrobial genes in thermophilic *Bacillus paralicheniformis* sp. associated with mobile elements

Ola Elsakhawy, Dr. Mohamed Abouelkhair, Dr. Stephen Kania, and Rebekah Jones

Bacteria in extreme environments adapt rapidly and produce an array of antimicrobials to inhibit other organisms as they compete for scarce resources. Thermal features represent one such environment and are useful to study of the development, evolution and transmission of antimicrobial genes. However, horizontal gene transfer from extremophile bacteria has been discounted due to a lack of association with mobile genetic elements. This study examined antimicrobial resistance genes and their genomic locations relative to transposons and bacteriophage in *Bacillus paralicheniformis* isolated from hot springs. Bacteria were collected in Yellowstone National Park during 2020. For propagation, samples were divided into three types of liquid media: malt yeast, ATCC Medium 1554, and peptone-yeast-glucose. Samples were incubated aerobically and anaerobically for 10 days. Broths positive for growth were subcultured to blood agar plates and bacterial colonies were isolated, DNA was extracted, and stocks were frozen. A strategy was developed to identify difficult to place elements including transposons, bacteriophage and plasmids. We used a short-read-first hybrid assembly method (short-read assembly followed by long-read bridging and polishing). Genes associated with resistance to bacitracin, beta lactamase and its accessory proteins, virginiamycin, oleandomycin, bicyclomycin, linearmycin, chloramphenicol and rifamycin were identified. Multiple copies of transposons were located in several positions. These transposons, which have been associated with horizontal gene transfer, were found in close proximity to clusters of resistance genes. These findings, as well as the identification of bacteriophage and plasmids suggest the potential for exchange of resistance genes between organisms in this extreme environment.

### Alterations in arthropod and neuronal exosomes reduce virus transmission and replication in recipient cells

Kehinde Damilare Fasae, Dr. Girish Neelakanta, and Dr. Hameeda Sultana

Our recent studies have shown that arthropod-borne flavivirus full-length RNA genomes and proteins (such as Envelope protein, Non-Structural protein 1 (NS1) and/or perhaps polyproteins) are transmitted to the vertebrate host cells via arthropod exosomes. Flaviviral transmission from the infected vectors (such as ticks and mosquitoes) to the vertebrate host via exosomes could be an important strategy for dissemination of these vector-borne pathogens. The aim of this current study is to target the modes of pathogen shedding/transmission via exosomes, which has been envisioned as a best approach to control vector-borne diseases. This study is focused on altering exosomes stability to affect the pathogen transmission from infected to naïve recipient cells. Our quantitative real-time PCR and immunoblotting analyses revealed that treatment of neuronal or tick exosomes at warmer temperatures of 37 °C or 23 °C, respectively, or with sulfate salts such as Magnesium or Ammonium sulfates or with highly alkaline pH of 9 or 11.5, would dramatically reduce transmission of a tick-borne Langat virus via infectious exosomes to naïve recipient human neuronal cells or human skin keratinocytes, respectively. Taken together, all the results from this study suggests that exosome-mediated viral transmission of vector-borne pathogens to the vertebrate host or the viral dissemination and replication within or between the mammalian host can be reduced by altering the ability of exosomes with basic changes in temperatures, salts or pH conditions. Overall, our study represents a way to interfere with the transmission of flaviviruses and perhaps other vector-borne pathogens. We believe that this is an important study that could change the way we think about the approaches and strategies to interfere with the modes of pathogen transmission from vector to human and other vertebrate hosts.

### A field trial of two point-of-care glucometers in healthy ewes

Ryan Flynn, Kailee Bennett, Jessie Gerber, Lainey Harvill, Olivia Escher, Dr. Jessica Garcia, Dr. Pierre-Yves Mulon, Dr. Lisa Ebner, and Dr. Joe Smith

**Abstract Background:** Several point-of-care (POC) glucometers are readily available for use by veterinary practitioners; however, the performance of certain veterinary marketed POC glucometers have not been evaluated for sheep. Because of this, practitioners are left with uncertainty when it comes to the performance or bias if using these devices in ovine patients. **Objective:** To compare the performance of a POC blood glucose (BG) measuring device validated for dogs and cats (Alphatrak 2; AT2) to a POC glucometer already validated for in healthy ewes (Precision Xtra; PX). **Animals:** Four healthy ewes had blood samples collected at various time points across 3 different days within a 3-week period. **Methods:** Once collected, blood samples were simultaneously evaluated by both devices. The AT2 analysis compared both the canine and feline settings. 88 blood samples were collected – 54 comparing the AT2 canine setting with the PX device and 54 comparing the AT2 feline setting with the PX. The results were evaluated via regression and Bland-Altman analysis. **Results:** Pearson R values for feline and canine settings were 0.7269 and 0.4710 respectively. With both canine and feline settings, the AT2 overestimated BG concentrations compared to the PX. The AT2 canine readings showed increased bias (canine bias:  $21.24 \pm 8.087$ ) compared to the AT2 feline readings (feline bias:  $14.54 \pm 5.878$ ). **Conclusions & Clinical Relevance:** Veterinarians should be aware of the bias if using the AT2, in either the canine or feline settings, for BG evaluation in sheep compared to the PX device.

### Development of a reverse transcription loop-mediated isothermal amplification (RT-LAMP) assay for rapid detection of canine distemper virus

Celia Gelpey, Dr. Mohamed Abouelkhair, Katie Dziendziel, and Aaron Shults

*Canine morbillivirus*, more commonly known as canine distemper virus (CDV) is an enveloped, negative sense single stranded RNA virus that causes severe disease in dogs and wildlife. Clinical diagnosis of CDV requires samples to be submitted to clinical laboratories for testing, most commonly via RT-qPCR which is costly and time consuming. An accurate point of care (POC) diagnostic test for CDV is needed to lower diagnostic costs and provide rapid results for use in the field and in private veterinary practice. In this study reverse transcriptase loop mediated isothermal amplification (RT-LAMP) assays were developed as a rapid, qualitative, POC molecular test for CDV diagnosis. Genetic material was extracted from clinical canine and wildlife samples of urine, EDTA whole blood, nasal secretions and tissues then underwent CDV molecular detection using RT-LAMP and RT-qPCR. RT-qPCR was used as a reference result. Three RT-LAMP primer sets were evaluated, with the primer set targeting the viral CDV phosphoprotein (p) gene showing the highest sensitivity, specificity and accuracy and the potential for use as an accurate POC molecular detection of CDV.

### Testing virulence of a *Leptospira* strain in hamsters: A preliminary study

Myranda Gorman, Dr. Liana Nunes Barbosa, Dr. Alejandro Llanes and Dr. Sree Rajeev

In this study, we tested the virulence of a laboratory adapted *Leptospira interrogans* serovar Copenhageni strain (SK1\_2022) isolated from a clinical dog in hamsters. Four-week-old hamsters were inoculated with different concentrations ( $10^3$ ,  $10^5$ ,  $10^8$ ) of the strain and were observed for 14 days. None of the hamsters developed any signs of active infection. Both culture and PCR of the kidney samples were also negative for the presence of *Leptospira*. Additionally, histologic examination of the lungs, liver, spleen, and kidneys found no lesions consistent with active *Leptospira* infection. Considering the potential loss of virulence during laboratory maintenance, we pursued whole genome sequencing on the isolate and compared it to the WGS of the original isolate (SK1\_2017). We observed a higher number of variants in SK1\_2022 (n=503) when compared to SK1\_2017 (n=117). In addition to the 117 variants originally found in SK1\_2017, we found 326 new variants of which 46 appear to affect annotated protein-coding genes with an expected high impact on the gene expression. Eighteen new high-impact and potentially inactivating mutations in SK1\_2022 may be associated with loss of virulence. We identified two metabolic enzymes, two membrane transporters, and two transcriptional regulators. A sensor histidine kinase and the transcriptional regulator of the LytR family are among the important genes inactivated by the mutations. However, approximately half of the genes affected by these mutations are of unknown functions. Our findings confirm that the genetic changes occurring during laboratory maintenance can affect the outcome of pathogenicity studies.

### Evaluation of safety and osteochondral activity after repeat intra-articular allogeneic stem cell injections in MHC-mismatched horses

Layla Gray, Dr. Liz Collar, and Dr. Madhu Dhar

Allogeneic bone Marrow mesenchymal stem cells (BM-MSC) are a promising treatment for musculoskeletal injuries in horses; however, controversy exists over the viability, efficacy, and safety in injecting major histocompatibility complex (MHC) mismatched BM-MSC as they have been demonstrated to cause antibody production in the recipient animal. However, MHC matched horses are very rare. This study will assess the safety of two allogeneic BM-MSC injections from two MHC-mismatched donors, as well as follow any cartilage and bone activity. Simultaneously, this study will evaluate the viability of donor and/or recipient cells after intraarticular allogeneic BM-MSC injection. Six control and six recipient horses without lameness at a walk will be utilized. Recipient horses will have either the left or the right front metacarpophalangeal joint (MCPJ) randomly selected for injection of 20 million BM-MSCs. The contralateral limb of the recipient horses will serve as an internal control. Control horses will have either the left or right MCPJ randomly selected for sterile saline (1.5mL) injection and synovial fluid (SF) sampling. SF and blood will be collected days 1, 7, 21, 28, and 42. Blood will also be collected 24 hours post injection (days 2 and 22). Biomarker protein assays (osteocalcin, CTX1, Co1 CEQ, CPII) will be performed on all serum and SF samples. SF will be plated and cultured until passage 3. Cultured cells will be sent for DNA analysis. Our hopes are to prove the use of MHC mismatched BM-MSC can be a safe and effective treatment for osteoarthritis in the horse.

## Relationship of placentome vascular perfusion and circulating pregnancy associated glycoproteins throughout gestation in pregnant beef heifers

Dr. Caroline Griffin, Dr. Andi Lear, Caleb Lemley, Ky Pohler, Piper Gauthier, and Sydney Campbell

During pregnancy, blood flow to the uterus changes to support fetal demand. Placentomes serve as vascular attachment sites for exchange of gases, nutrients, and metabolic products. Non-invasive methods of ultrasonography and biomarkers have been described to assess placental health and fetal viability. Pregnancy associated glycoproteins (PAGs) are produced by the ruminant placenta and detected in maternal circulation. Changes in circulating PAG concentrations may be a useful biomarker for assessing placental health. The objective of this study is to determine the association between placentome blood perfusion and circulating PAG concentrations as they relate to the health of the developing fetus. The hypothesis states that placentome perfusion and PAG concentration will be positively correlated and associated with neonatal outcome. A prospective, observational study was designed using 30 pregnant, nulliparous, Angus heifers and variables assessed throughout gestation. Placentome blood perfusion was visualized monthly via transrectal Doppler ultrasonography with power flow function. Ultrasound images were analyzed using ImageJ software to determine the percent area of perfusion and integrated pixel densities. Venous blood was collected monthly. PAG concentrations were determined via a commercially available serum PAG enzyme-linked immunoassay. Following parturition, calving characteristics were assessed. Preliminary results indicate that mean placentome blood perfusion increases as gestation advances. PAG concentrations demonstrated the expected temporal trend, increasing with gestation length, and were positively linearly correlated with placentome perfusion. The relationship identified between circulating PAG concentration and placentome blood perfusion validates the use of transrectal power flow Doppler ultrasonography as a noninvasive technique to determine placental blood flow morphometrics.

## Evaluating chemokine and sphingolipid receptor expression in canine diffuse large B cell lymphoma (DLBCL)

Anna Hauck, Dr. Brandy Kastl, and Dr. Nora Springer

Introduction: Lymphomas are the most common blood cancer in dogs. Canine lymphomas have survival times of approximately one year with chemotherapy treatment. A low percentage of patients have sustained clinical remissions. Current prognostic methods, immunophenotype or histological subtype, are unable to predict which patients might respond more favorably to treatment. Accordingly, there is need for biomarkers that predict which patients will benefit from chemotherapy to achieve sustained cancer remission. In people, expression patterns of chemokine receptor CXCR4 and sphingolipid receptors S1PR1 and S1PR2 are associated with prognosis in several forms of lymphoma. Thus, chemokine and sphingolipid receptor expression might also be prognostic in canine lymphoma. Objective: Determine whether the most common form of canine lymphoma, DLBCL, expresses CXCR4, S1PR1 and S1PR2. Methods: Immunohistochemistry using antibodies validated for canine CXCR4, S1PR1 and S1PR2 was performed on 87 archived cases of formalin-fixed and paraffin embedded canine DLBCL. Immunoreactivity was scored as weak, moderate, or strong by a rubric incorporating both staining intensity and percent positive cells. Results: All three receptors were detected in canine DLBCL. Receptor expression was variable across cases. CXCR4 had 7% weak, 34% moderate, and 59% strong staining. S1PR1 had 40% weak, 39% moderate, and 21% strong staining. S1PR2 had 28% weak, 41% moderate, and 31% strong staining. Conclusions: The variability in CXCR4, S1PR1 and S1PR2 staining across canine DLBCL cases suggests that these receptors might have utility as a biomarker. Prospective studies are necessary to correlate expression pattern with clinical outcomes in dogs with lymphoma.

## Chronic obstructive pulmonary disease (COPD) among former Department of Energy workers in the United States, 2006-2019

Sara Howard and Dr. Agricola Odoi

Background: Chronic obstructive pulmonary disease (COPD) is a chronic inflammatory lung disease, which reduces lung function and primarily affects older adults. Evidence suggests that occupational exposures like diesel exhaust, cadmium, welding fumes, and silica increase the risk of COPD. Historically, the United States Department of Energy (DOE) workers participated in activities that may result in exposures to these noxious substances. Identification of factors associated with COPD is imperative for improving control and preventive efforts. Therefore, the objectives of this study were to estimate COPD burden among DOE former workers and investigate occupational predictors of COPD while controlling for other risk factors. Methods: Data from the National Supplemental Screening Program for former DOE workers were obtained for 2006-2019. Multivariate imputation by chained equation was used to impute missing values. Binary and multinomial logistic regression models were used to investigate predictors of COPD occurrence and severity, respectively. Results: Of the 17,960 study participants, 21.3% had COPD. History of asthma, age at exam, body mass index, and duration of smoking were significant predictors of both COPD occurrence and severity. Individuals exposed to silica had higher odds of COPD compared to those that were not exposed to silica. Similarly, diesel exhaust exposure was significantly associated with risk of more severe COPD. Conclusions: These findings are important for: (a) understanding how occupational exposures influence disease occurrence and severity; (b) guiding control and preventive programs.

## Pharmacokinetics and pharmacodynamics of intravenous and oral esomeprazole in horses

Rebekah Johnson, Dr. Liz Collar, and Dr. Joe Smith

Equine Gastric Ulcer Syndrome is a common condition, leading to issues such as poor performance, colic, and even death. Omeprazole, the standard treatment, is not available as an intravenous formula in the US. Horses unable to take oral medications need an intravenous option. Esomeprazole is available as an IV formula and has an effective clinical profile on raising gastric pH. This randomized crossover study used 6 female horses and had a PK and PD phase, utilizing a 0.5mg/kg dose of IV or oral esomeprazole. The PK study had a 24-hour sample period with 19 blood samples. After a 7-day washout the horses received the opposite treatment. The first PD phase spanned over 7 days, with each horse receiving a total of 6 doses of 2g phenylbutazone and 4 doses of 10mg dexamethasone, alongside two doses of esomeprazole. Gastric pH was assessed daily, and their ulcer scores were captured with gastroscopy. Oral esomeprazole results: C<sub>max</sub>, T<sub>1/2</sub>, AUC and F: 104 ng/mL, 40 min, 114 ng/mL×hr and 25%. IV esomeprazole results: C<sub>0</sub>, T<sub>1/2</sub>, and AUC as follows: 2030 ng/mL, 24 min, and 660 ng/mL×hr. The pharmacodynamic results have shown ulcer formation from phenylbutazone and dexamethasone, with no evidence of healing with esomeprazole administration. Both routes of esomeprazole raised the gastric pH by 48 hours of use. Pharmacokinetic data could help clinicians discover ideal dosages and dose intervals for esomeprazole. Pharmacodynamic data may help advise veterinarians on the proper use of esomeprazole alongside phenylbutazone and dexamethasone.



### Fecal float and examination of tissue samples for parasites of black bears (*Ursus Americanus*)

Monica Lee, Dr. Rick Gerhold, and Katie Riese

American black bear (*Ursus americanus*) populations in the eastern Tennessee and North Carolina regions have been growing over the past decades. With the increasing population density, there is concern by wildlife and veterinary professionals of increased spread of diseases. This is a concern for not only bear health, but also public health. Research on the health of the black bears in this region is limited. During this study, we obtained blood, feces, skin scrapes, swabs from the oral, nasal, and rectal regions, and ectoparasites, if present, from sixty-eight bears. A complete blood count (CBC), organ chemistry, Knott's test, fecal float, and skin cytology were performed on the appropriate samples. Of the 60 bears that were tested for microfilaria through Knott's tests, 50 bears (83.3%) were positive. Identification of the microfilaria is pending. *Baylisascaris transfuga* and various arthropods have been identified in the fecals thus far. Two species of ticks have been morphologically identified: *Dermacentor variabilis* and *Amblyomma maculatum*. The results of this study emphasize the need for further disease surveillance of wild bear populations. This research will help provide wildlife and veterinary professionals a baseline health assessment of the black bears of this region; therefore, the results can be used in the future to help these professionals better manage these populations and continue protecting the health of the public.

### An endosymbiont of a relapsing fever tick *Ornithodoros turicata*

Lichao Liu, Dr. Daniel E. Sonenshine, Dr. Hameeda Sultana, and Dr. Girish Neelakanta

Endosymbiotic bacteria generally exist in both hard and soft ticks. They participate in many important biological processes including growth and survival of tick hosts and in acquisition and transmission of pathogens from them. *Occidentia massiliensis* is the first and the only identified species in the *Occidentia* genus that belongs to the family of *Rickettsiaceae*. This bacterial presence was confirmed in a soft tick *Ornithodoros sonrai* in Senegal and a hard tick *Africaniella transversale* in the United Arab Emirates. In this study, a species genetically similar to *Oc. massiliensis*, designated as *Occidentia*-like species, was found in a relapsing fever tick *O. turicata*. Two genetic markers, 16S rRNA and groEL were used for the confirmation of the presence of *Occidentia*-like species via sequencing and phylogenetic analyses. The *Occidentia*-like species was present in all developmental stages of *O. turicata* including eggs. This bacterial presence was noted in different tick tissues and at a significantly high level in the ovaries compared to the gut. In addition, the expression of *Occidentia*-like species groEL was significantly high in synganglion compared to the ovary and the gut. Furthermore, blood feeding leads to a significant increase in the level of *Occidentia*-like species in *O. turicata* ticks. In summary, this study identified *Occidentia*-like species as an endosymbiont of *O. turicata* and suggested a potential role of this bacterium in tick-endosymbiont interactions.

## Hematology and plasma chemistry comparisons among juvenile American black bears (*Ursus americanus*) undergoing rehabilitation

Ally Mayhew, Dr. Luca Giori, Dr. Xiaojuan Zhu, and Dr. Julie Sheldon

The American black bear (*Ursus americanus*) is an opportunistic and adaptable wildlife species with high rehabilitation success rates. Injured, ill, and orphaned bears across the southeastern United States are examined and treated at the University of Tennessee College of Veterinary Medicine (UTCVM) followed by rehabilitation at Appalachian Bear Rescue (ABR). Reference ranges of hematology and biochemistry parameters exist for adult black bears; however, most bears presenting to ABR are young and of variable health status. Thus, further investigation into the difference of blood parameters at varying ages and presentations is warranted. ABR records from 1996-2022 include 109 bears with completed bloodwork (22 paired samples at intake and release, 87 intake-only). Intake-only samples consisted of 12 neonates (<3mo old), 67 cubs (3-12mo), and 30 yearlings (1-2yr). Bears presented as orphaned neonates (21%), orphaned cubs (45%), malnourished yearlings (23%), and injured/ill (11%) during fall (17%), winter (13%), spring (39%), and summer (31%). Changes in hematology and plasma chemistry between intake and release included an increase in hematocrit and glucose. Injured/ill bears presented with significantly higher total WBC, neutrophils, ALT, AST, and CK. Positive correlations between ALT, AST, proteins, and BUN, and negative correlation between lymphocytes and ALP were noted with age. Both total WBC and neutrophil counts were significantly lower during winter. Understanding what factors affect bear blood parameters improves clinical expectations and evaluation upon intake, clinical evaluation and treatment.

## *Leptospira* Seroprevalence in dogs and cats in Tennessee

Kellie McCreight, Dr. Liana Nunes Barbosa, and Dr. Sree Rajeev

Introduction: Leptospirosis is a re-emerging zoonotic disease in humans and animals. It is estimated that there are more than 1 million new human cases worldwide per year, with almost 60,000 deaths. Leptospirosis can be life threatening in canines and is an emerging disease in cats. The purpose of this study was to investigate the *Leptospira* seroprevalence in dogs and cats in Tennessee. Methods: We collected convenient serum samples from dogs and cats submitted to the UTCVM diagnostic laboratory. The samples were tested for leptospirosis by Microscopic Agglutination Test (MAT) against twelve *Leptospira* serovars. Results: The overall *Leptospira* seroprevalence was 29.44% (111/377) and 12.43% (21/169) in dogs and cats, respectively. The highest seroprevalence in dogs was against serovar Autumnalis (73.87%). In cats, the highest seroprevalence was against serovar Bratislava (50%). The titers ranged from 1:50 to 1:1600 in canines with the highest titers being against serovar Bratislava. The titers ranged from 1:50 to 1:3200 in felines, with the highest titer being against serovar Hardjo. PCR and sequencing are being performed on a random set of canine and feline urine samples to assess the presence of renal infection and to determine the circulating serovars and species. Evaluation of clinical samples submitted for diagnostic testing identified seven positive samples out of twenty-seven (25.93%). Our preliminary analysis by conventional PCR and sequencing samples identified *Leptospira kirschneri* as the infecting species in dogs. Conclusion: Our study concludes that dogs and cats are exposed to *Leptospira* and canine leptospirosis is not an uncommon disease.

### Local anesthetic delivery via an indwelling retrobulbar catheter in horses

Dr. Leah Moody, Dr. Braidee Foote, Dr. Diane Hendrix, and Dr. Dan Ward

Purpose: To evaluate the effects of local anesthetic delivery via an indwelling retrobulbar catheter on corneal sensitivity, pupillometry, and ocular motility in normal horses. Methods: One eye was randomly selected from seven healthy horses. A 20-gauge long-line catheter was placed in the retrobulbar space and injected with either 10mL of 0.5% bupivacaine HCl or 0.9% sodium chloride. Cochet-Bonnet esthesiometry (CBE), pupil photogrammetry, pupillary light responses (PLRs), and oculocephalic reflexes were evaluated prior to the injection (t=0) and at t=15min, 1, 3, 6, 9, and 12 hours after injection. Following a 7-13 day washout period, this procedure was repeated using the injection solution that was not used previously. Corneal touch thresholds (CTTs) derived from CBE and pupillary areas (PA; as measured from photographs) were compared across time for each group. PLRs and oculocephalic reflexes were compared between groups at each evaluation time point. Results: Injection of 0.9% sodium chloride did not significantly affect CBE, PA, PLRs, or oculocephalic reflex at any time point. Injection of 0.5% bupivacaine HCl significantly reduced CTT ( $P<0.001$ ) for 6 hours and increased PA ( $P=0.037$ ) for 3 hours. PLRs and oculocephalic reflexes were maintained following saline injection at all time points. Following bupivacaine injection, PLRs were either reduced or absent for nine hours and oculocephalic reflexes were reduced for three hours. Mild adverse effects included chemosis, blepharoeedema, and transiently reduced palpebral reflex. Conclusions: Injection of bupivacaine via an indwelling retrobulbar catheter in horses reduces corneal sensitivity and may be useful in treating horses with corneal disease.

### Bacterial contamination of the environment of veterinary rehabilitation clinics

Nick Millis, Dr. David Levine, Dr. Darryl Millis, and Dr. Henry Spratt

Background: The presence of potentially pathogenic bacteria on surfaces in veterinary clinics is problematic for animals and people. Animals in these clinics often touch contaminated surfaces with their feet, noses, and mouths. Studies of Methicillin-resistant *Staphylococcus aureus* (MRSA) present the possibility of pets harboring MRSA contracted from humans that could lead to cross-transmission back to humans. The focus of this study was to determine the contamination of environmental surfaces by potentially pathogenic bacteria in five veterinary rehabilitation clinics. Methods: Sampling involved using 30 double transport swabs from 13 different locations in each clinic. The two swabs were used to inoculate multiple types agar plates for different bacterial species. Results: The most prominent species cultured from the clinics was *C. diff*. Enteric bacteria were the second most encountered bacteria. Both MRSA and SIM were found on approximately 10% of swabs collected. Of the clinic sites sampled, the largest number of positive swabs were from the floor and air ventilation sites. Four water samples were collected from the underwater treadmills. Conclusions: *C. diff* was the most prominent bacterial species on environmental surfaces in these clinics, with clinic floors and HVAC systems having the highest levels of contamination. SIM tends to be more pathogenic for dogs, and notable levels of *Staphylococcus* species throughout the clinics sampled. Targeted cleaning and disinfecting, along with frequent monitoring of veterinary rehabilitation facilities, may reduce risks of infection in both animals and humans in clinics.

### Rickettsial pathogen uses arthropod tryptophan metabolite xanthurenic acid to facilitate tick cell survival

Dr. Prachi Namjoshi, Dr. Mustapha Dahmani, Dr. Hameeda Sultana, and Dr. Girish Neelakanta

*Anaplasma phagocytophilum*, an obligate intracellular rickettsial pathogen, is a causative agent of human anaplasmosis and a blacklegged tick, *Ixodes scapularis*, serves as a primary vector for this pathogen. In this study, we provide novel evidence that *A. phagocytophilum* uses arthropod tryptophan metabolite, xanthurenic acid (XA), to increase survival of ticks by activation of p38 mitogen activated kinase. We found that *Anaplasma phagocytophilum*-infected ticks had significantly increased levels of XA compared to the levels noted in uninfected ticks. *Anaplasma phagocytophilum*-infected tick cells exhibited significantly increased levels of total and phosphorylated p38 MAPK in comparison to the levels noted in uninfected controls. In addition, *A. phagocytophilum*-infected tick cells treated with XA exhibited decreased cell death markers and increased phosphorylated p38 MAPK levels as compared to mock-treated controls. *Anaplasma phagocytophilum*-infected tick cells when treated with BIRB796, p38 MAPK inhibitor, exhibited decreased bacterial load, decreased phosphorylated p38 MAPK levels and increased cell death. However, these BIRB796 inhibitor-induced effects were reversed in the presence of XA. Altogether, this study demonstrates how a rickettsial pathogen increases the survival of the vector host via modulation of the arthropod tryptophan and p38 MAPK pathways.

### Subolesin regulates innate immune genes in soft ticks

Krittika Nandy, Comfort Tamakloe, Dr. Daniel E. Sonenshine, Dr. Hameeda Sultana, and Dr. Girish Neelakanta

Hard and soft ticks are obligate, hematophagous arthropods that vector and transmit several pathogens to the humans. Recent data indicates that there has been a marked rise in the incidence of tick-bite events and tick-borne diseases. *Ornithodoros turicata* is a soft tick that transmits relapsing fever spirochete *Borrelia turicatae*. There are no vaccines to treat or prevent tick-borne relapsing fever in humans. Recent studies have highlighted consideration of vector molecules as candidates in the development of anti-vector vaccine to treat/prevent several tick-borne diseases. In our previous study, we identified an anti-vector vaccine candidate, subolesin, in *O. turicata* ticks. In this study, we characterized several innate immune genes including Toll, ML-domain protein (MLDP), fibrinogen-domain-containing protein (FDP), Lysozyme precursor and Cystatin in *O. turicata* ticks. QRT-PCR analysis show variable gene expression of these genes in different *O. turicata* developmental stages. RNAi-mediated knockdown analysis followed by feeding and measurement of tick body weights revealed significantly reduced blood digestion in *subolesin*-dsRNA treated ticks. In addition, we noted significantly reduced expression of all the analyzed *O. turicata* innate immune genes in *subolesin*-dsRNA-treated ticks compared to the levels noted in mock-dsRNA-treated controls. Collectively, these results show that subolesin not only facilitates blood digestion but also regulates innate immune gene expression in soft ticks.

### The molecular epidemiology of *S. pseudintermedius* isolated from dogs in South Africa

Lufuno Phophi, Dr. Mohamed Abouelkhair, Rebekah Jones, Dr. Maryke Henton, Dr. Nenene Gekwana, and Dr. Stephen Kania

*Staphylococcus pseudintermedius* is reported as a cause of clinical infections in small-animal-veterinary medicine. Genetic evolutionary changes have been observed among methicillin-resistant *S. pseudintermedius* (MRSP) strains in most continents, however, there are no studies on the antimicrobial resistance and clonal lineages of MRSP in South Africa. Therefore, this study aimed to determine the molecular epidemiology of *S. pseudintermedius* isolated from dogs in South Africa. Non-duplicate, clinical isolates from dogs were obtained as convenience samples from a veterinary diagnostic laboratory in Gauteng, South Africa. Twenty-three isolates were confirmed as *S. pseudintermedius* by MALDI-TOF, of which 14 were MRSP. In addition to beta-lactam antimicrobials, MRSP isolates were resistant to tetracycline (85.7%), doxycycline (92.8%), kanamycin (92.8%), and gentamicin (85.7%) using the disk diffusion method. The isolates harbored antibiotic resistance genes (*tetM*, *ermB*, *drfG*, *cat*, *aac6-aph2*, *ant6-la*, *aph3-III*) and virulence genes (*AdsA*, *geh*, *icaA*, *lip*). Eight isolates in this study had novel sequence types (ST2228, ST2229, ST2230, ST2231, ST2232, ST2318, ST2326 and ST2327). Previously reported STs such as ST45, ST71, ST181, ST551, and ST496 were also identified. This is the first study reporting on the characterization of MRSP and STs in South Africa. The detection of highly resistant strains of *S. pseudintermedius* in South Africa is a public health concern and warrants more comprehensive studies to elucidate the molecular epidemiology and the changing population structure of *S. pseudintermedius* in South Africa.

### Organic anion transporting polypeptide based anti-vector vaccine impairs transmission of human anaplasmosis agent from ticks

Dr. Mahesh Puthiyottu Poyil, Dr. Prachi Namjoshi, Dr. Hameeda Sultana, and Dr. Girish Neelakanta

Anaplasmosis is a major tick-borne disease in humans caused by the bacterium, *Anaplasma phagocytophilum*. There is no vaccine approved against this disease to date. Previous studies from our laboratory showed that the *Ixodes scapularis* organic anion transporting polypeptide 4056 (IsOATP4056) was upregulated in *A. phagocytophilum*-infected ticks and tick cells that was critical for the survival of this bacterium in the vector host. In this context, this study rationalizes on targeting IsOATP4056 to impair transmission of this bacterium from ticks to vertebrate hosts. The membrane bound IsOATP4056 has six extracellular loops, out of which the sixth one is the longest one and completely exposed out of the membrane. Two affinity purified rabbit polyclonal antibodies raised against epitopes in extracellular loops two and six (EL-2 and EL-6) were utilized in this study. ELISA, immunoblots, and immunofluorescence analyses show increased expression of IsOATP4056 in *A. phagocytophilum*-infected ticks and tick cells. Treatment with high dose (10 µg/ml) but not low dose (5 µg/ml) of EL-6 antibody in tick cells showed cytotoxicity but not in human keratinocyte cell line (HaCaT). Mice that were passively immunized with EL-6 antibody showed significantly reduced loads of the bacteria in both blood and spleen when *A. phagocytophilum*-infected ticks were fed on them. In addition, arthropod Toll pathway was upregulated in both ticks and tick cells in the presence of EL-6 antibody. Furthermore, reduced molting efficiency was noted in ticks fed on EL-6 antibody immunized mice. Taken together our study provides evidence on a novel candidate for the development of vaccines against ticks.

## Clinical and gastrointestinal changes in healthy research dogs administered prednisone, prednisone/omeprazole, or prednisone/probiotics

Dr. Mariola Rak, Tamberlyn Moyers, Joshua Price, and Dr. Jacqueline Whittemore

Background – Previously evaluated gastroprotectants did not prevent glucocorticoid-induced gastric bleeding in dogs, but neither probiotics or twice-daily omeprazole were evaluated.

Hypothesis/Objectives – Compare gastrointestinal bleeding among dogs administered prednisone, prednisone/omeprazole, or prednisone/probiotics.

Animals – Twenty-four healthy research dogs.

Methods – Double-blinded, placebo-controlled randomized trial. Dogs received placebo, prednisone (2mg/kg q24h), prednisone/omeprazole (1mg/kg q12h), or prednisone/probiotics (11.2-22.5 billion CFU/kg q24h) for 28 days. Clinical signs and endoscopic gastrointestinal mucosal lesions were determined at baseline (t1), day 14 (t2), and day 28 (t3). Results were compared using split-plot repeated measures mixed-model ANOVAs.

Results – Attitude, vomiting, and food intake did not differ among groups. Fecal score differed by treatment-by-time ( $F [6,40] = 2.65$ ,  $P < .029$ ), with increased scores in prednisone-receiving groups between t1 and t3 ( $P < .001$  for each). Nineteen of 33 (58%) instances of diarrhea occurred in the prednisone/omeprazole group. Gastric mucosal lesion scores differed by treatment-by-time ( $F [6,60] = 2.86$ ,  $P = .016$ ), between treatments ( $F [3,60] = 4.9$ ,  $P = .004$ ), and over time ( $F [2,60] = 16.5$ ,  $P < .001$ ). Post-hoc analysis revealed lesion scores increased over time for prednisone-receiving groups ( $P \leq .03$ ). Although scores for the prednisone/omeprazole group increased over time, no difference was observed between prednisone/omeprazole and placebo at t2 or t3 ( $P \geq .26$ ). Ulcers occurred only in prednisone-receiving dogs.

Conclusions and clinical importance – Consistent with previous studies, prednisone induced gastric bleeding. Co-administration of omeprazole partially mitigated bleeding, but a similar protective benefit was not demonstrated by co-administration of probiotics.



### Effects of acute endotoxin inflammation on reproductive neuroendocrinology in intact ewes

Allison Renwick, Hannah Sylaidis, Dr. Brian Whitlock, Dr. Casey Nestor, Dr. Rebecca Payton, and Dr. Lannett Edwards

Systemic inflammation is a common ailment in various diseases and infections, oftentimes caused by endotoxin from gram negative bacteria such as E coli. Inflammation and immune response impair reproduction, however the full mechanism(s) has yet to be uncovered. In this experiment, nine ewes were randomly assigned into control (CON; 0.9% NaCl IV; n=4) or endotoxin (ENDO; 400 ng of lipopolysaccharide / kg of body weight IV in 0.9% NaCl; n=5) groups. All ewes underwent estrus synchronization to ensure proestrus for the experiment. Blood samples were collected every 12 minutes for 8 hours for luteinizing hormone (LH) and progesterone concentrations. Temperature was recorded hourly using vaginal temperature probes. Following the final blood sample, ewes were humanely euthanized and brain and pituitary tissues were fixed and collected. Tissue was sectioned and underwent immunohistochemistry for kisspeptin and cFos (hypothalamus) as well as LH $\beta$  (anterior pituitary). Vaginal temperature increased over time following endotoxin administration with significantly greater mean vaginal temperature in ENDO compared to CON starting 2 hours post treatment and continuing to the end of blood collection. There was an increase in mean progesterone concentration over time in ENDO while CON remained at 0 ng/mL for all timepoints. There was no effect on the number of LH $\beta$  immunopositive cells. Kisspeptin immunopositive cell quantification and LH concentration analysis are underway. Overall, acute endotoxin exposure initiates an immune response and potentially impairs reproduction through suppression of the kisspeptin system.

### Gopher tortoise health assessment

Kendra Rich, Dr. Rebecca Hardman, Dr. Deb Miller, Kim Sash, Deanna Riente, and Dr. William Sutton,

The gopher tortoise (*Gopherus polyphemus*), a keystone species of the longleaf pine ecosystem, is currently experiencing a population decline. The primary threats impacting this population are disease and habitat loss. Gopher tortoises are combating an upper respiratory tract disease caused by a combination of Mycoplasma, Ranavirus and Testudinid Herpesvirus as well as significant habitat loss resulting from urban development and improper land management. Conservation efforts have responded to habitat loss by translocating tortoises to areas with established populations. Many research projects have followed the health status of tortoises following translocation, however, baseline health data for tortoise populations of recipient sites are lacking significantly. The objective of this study was to conduct a health assessment of tortoise populations at sites being considered for recipient sites. Tortoises were trapped and caught opportunistically at two sites located on Tall Timbers property in Tallahassee, Florida. Biometric data, blood samples, oral and cloacal swabs and fecal samples were obtained from 39 tortoises. Tortoises sampled included 56.4% adult and subadult males, 25.6% adult and subadult females, and 17.9% juveniles, unable to be sexed by secondary sex characteristics. Blood samples and swabs were submitted for multi-pathogen qPCR and results have not yet been acquired. Blood smear analysis indicated *Anaplasma* is present in the population. Clinical signs associated with respiratory disease were observed in six tortoises while ocular disease was observed in four tortoises. This research is being continued by Tall Timbers Research Station in accordance with Florida Fish and Wildlife Conservation Commission.

### Novel methods of immunogenic antigen selection for serological diagnosis of *Parelaphostrongylus tenuis* infection

Dr. Jessie Richards, Dr. Stephen Kania, Abigail Wilson, Emily Kent, and Dr. Rick Gerhold

This presentation outlines the methods used to identify novel antigens for serological diagnosis of *Parelaphostrongylus tenuis* infections in cervid hosts. Proteins extracted from *P. tenuis* organisms were affinity isolated using antibodies enriched from seropositive moose (*Alces alces*). The proteins were analyzed using mass spectrometry and liquid chromatography to obtain amino acid sequences that were then cross-referenced to open reading frames predicted from an assembled transcriptome. An antigen of interest was assessed for immunogenic epitopes and subsequently synthesized into 10-mer synthetic overlapping peptides representing these regions. These synthetic peptides were then assessed for reactivity against positive and negative moose sera and demonstrated potential use as a serological assay in diagnostic laboratories. Known negative moose sera revealed significantly lower optical density when compared to the positive samples ( $p < 0.05$ ). This method serves as a pipeline for the construction of diagnostic assays of pathogens in both human and veterinary medicine.

### Black bear population health monitoring in Eastern Tennessee

Katie Riese, Dr. Rick Gerhold, Dr. Eliza Baker, and Monica Lee

Recent growth of the American black bear (*Ursus americanus*) population in eastern Tennessee raises concerns about the potential spread of density-dependent diseases among bears. However, research on the health of bears in this area is limited. We analyzed samples from 149 bears in the region. We performed Knotts tests, skin scrapes, fecal floats; *Toxoplasma*, CDV, CAV, and CPV serology, and *Hepatozoon*, *Babesia*, *Trypanosoma*, and *Ehrlichia* PCR, and identified ectoparasites. We found that 82% (46 of 56) had microfilaria; species identification is pending. We performed 95 fecal floats and found 12 (12.6 %) had arthropods, seven (7.4%) had nematode larva, 14 (14.7%) had *Baylisascaris transfuga*, two (2%) had *Ancylostoma* spp., one (1%) had *Cryptosporidium* spp., one (1%) had unidentifiable coccidia, and one (1%) had *Eimeria* spp. We identified *Ursicoptes* spp. mites on two skin scrapes (3%). *T. gondii* antibodies were detected in 58.6% (41 of 70) of bears. CAV, CDV, and CPV serology was performed on 51 samples of which we had one CAV positive (2%), 10 CDV positives (19.6%), and one CPV positive (2%). Of the 25 bears tested, four were PCR-positive for *Babesia* (16%). All bears were PCR-negative for *Hepatozoon* spp., *Trypanosoma* spp., and *Ehrlichia* spp. Collected ticks were morphologically identified; 100% of bears (54 of 54) were infested with *Dermacentor variabilis*, and 9% (5 of 54) were infested with *Amblyomma maculatum*. This research will lay the foundation for future research on bear diseases in the southeast and aid wildlife managers with management decisions on free-ranging black bear populations.

Molecular characterization of thirteen <i>Staphylococcus pseudintermedius</i> strains isolated from the United States
Ashkan Roozitalab, Ola Elsakhawy, Dr. Stephen Kania, Rebekah Jones, and Dr. Mohammed Abouelkhair
<p><i>Staphylococcus pseudintermedius</i> is a Gram-positive, coagulase positive bacteria present as a skin and mucosal commensal in healthy dogs. While dogs are the main species to get afflicted with it, in humans it is present as an opportunistic pathogen that can create biofilms, secrete immune modulating virulence factors, and it is also known to possess adhesion factors but variability of genotypic features such as molecular epidemiology, biological characters, virulence and phenotypic characteristics have not been studied at length in MRSP isolates. In this study we sequenced and molecularly characterized thirteen <i>S. pseudintermedius</i>. Sequencing of isolates were performed using Illumina MiniSeq. De novo assemblies were performed by CLC Genomics Workbench. AMRFinderPlus tools from NCBI were used to annotate bacterial genomes and identify the AMR genes. Geneious Prime® tBLASTn function, SCCmecFinder 1.2, and spaTyper 1.0 tools were used for Agr typing, SCCmec types, and SPA identification, respectively. Additionally, PubMLST.org was used for Multi-locus sequence typing. The mean genome coverage, average genome completeness and average genome size for isolates were 20x, 99.43% and 2.62 Mbp, respectively. Staphylococcal <i>spsQ</i> was not identified in all isolates. AMR gene, <i>mecA</i>, was identified in 7 out of 13 (53.8%) isolates, and 6 out of those 7 isolates' SCCmec type were determined. Also, the <i>mecA</i>-carrier isolates contain comparatively greater antimicrobial resistant genes. WGS of drug-resistant isolates across the US causing infection in dogs and humans gives deep insights into the molecular epidemiology and biological characteristics of <i>S. pseudintermedius</i> and allows for further comparative genomic studies to understand bacterial pathogenesis.</p>

Evaluation of the perioperative analgesic effects of grapiprant compared with carprofen in dogs undergoing elective ovariohysterectomy
Dr. Juliet Ross, Dr. Stephanie Kleine, Dr. Christopher Smith, Dr. Becky DeBolt, Dr. Jennifer Weisent, Emma Hendrix, and Dr. Reza Seddighi
<p><b>Objective:</b> To evaluate and compare post-operative analgesic effects of grapiprant and carprofen in dogs undergoing ovariohysterectomy.</p> <p><b>Animals:</b> Forty-two sexually intact female, healthy dogs (&lt; 35 kgs and 0.5 – 7 years old) were enrolled.</p> <p><b>Procedures:</b> In a masked, randomized, non-inferiority clinical trial, dogs received either 2 mg/kg grapiprant or 4.4 mg/kg carprofen orally two hours prior to ovariohysterectomy. Post-operative pain was assessed using the Glasgow Composite Pain Scale – short form (GCPS-SF) and an algometer at extubation and 2, 4, 6, 8, and 18 hours post-extubation and compared to the baseline. After each pain scoring, mechanical nociceptive testing (MNT) with von Frey monofilaments was performed to assess hyperalgesia. MNT assessments were made 2 cm from the incision. Hydromorphone (0.05 mg/kg, intramuscular) was administered to any dog with a GCPS-SF of <math>\geq 5</math> out of 24. Data were analyzed with a mixed-effect ANOVA and post hoc comparisons were made with Tukey's correction method (<math>p &lt; 0.05</math>).</p> <p><b>Results:</b> Three dogs required rescue analgesia and were excluded from statistical analysis. Of the remaining 39 dogs; 18 received carprofen and 21 received grapiprant. There was no difference between treatment (<math>p=0.89</math>) nor treatment by time (<math>p=0.62</math>) for GCPS-SF or algometry measurements. Dogs in both groups had higher GCPS-SF scores at <math>t_0</math>, <math>t_2</math>, <math>t_4</math>, and <math>t_6</math> time points regardless of treatment. There was no difference between groups at any time point or over time when von Frey monofilaments were used.</p> <p><b>Clinical Relevance:</b> Our study results support the use of grapiprant as an analgesic alternative to carprofen in dogs undergoing ovariohysterectomy.</p>

## Violative levels of antibiotic residues in U.S. slaughtered food animals: Has the veterinary feed directive regulations changed the course?

Shamim Sarkar and Dr. Chika Okafor

The presence of antibiotic residues in animal tissues is a growing concern among consumers. An inspector-generated sampling dataset from the United States National Residue Surveillance Program, collected between 2014 and 2019, was analyzed to investigate the effect of veterinary feed directive (VFD) regulations on the detection of violative levels of penicillin, tetracycline, and sulfonamide residues in tissues of food animals. Multivariable logistic regression models were used for analysis. While the type of animal and type of tissue sampled were significantly associated with residue violations for both penicillin and tetracyclines, having a sample collection date after the implementation of VFD regulations was not. However, the odds of detecting violative levels of sulfonamide residues in food animal tissues sampled following the implementation of VFD regulations were 36% lower than those collected before the VFD regulation period, irrespective of animal type. Further investigation of the factors that influence the presence of violative levels of penicillin and tetracycline residues in the tissues of food animals following the implementation of VFD regulations would lend clarity to this critical issue.

## Pharmacokinetics of oral clonazepam in commercial swine (*Sus Scrofa*)

Alex Shanks, Dr. Stephanie Kleine, Dr. Chiara Hampton, Dr. Christopher Smith, Dr. Pierre-Yves Mulon, Dr. Reza Seddighi, Dr. Sherry Cox, and Dr. Joe Smith

Clonazepam is a benzodiazepine used for the treatment of panic disorders and anxiety in humans and as an anti-convulsant in dogs. This drug has also been shown to produce sedation and psychomotor impairment in people. The objective of this study was to determine the pharmacokinetics (PK) of oral clonazepam as a first step in the evaluation of clonazepam as an orally administered tranquilizer in pigs. Eight healthy, growing commercial swine (3 barrows and 5 gilts) aged 91 days and weighing  $43.8 \pm 2.4$  kg received oral clonazepam (0.5 mg/kg) after food withheld for 12 hours. Serial blood sampling from an indwelling jugular catheter was performed before treatment administration and at pre-established timepoints for up to 96 hours for PK analysis. Harvested plasma was stored at  $-80^{\circ}\text{C}$  until clonazepam analysis using reverse phase high-performance liquid chromatography and analyzed with a commercial PK software. A non-compartmental model was used to describe time-concentration PK data. The mean maximum plasma concentration ( $C_{\text{max}}$ ) was 97.3 ng/mL and was reached in 3 hours ( $T_{\text{max}}$ ). Terminal elimination half-life and mean resident time were 5.6 and 10.5 hours, respectively. A large volume of distribution ( $V_z/F$ ; 4.2 L/kg) was observed. Sedation lasting approximately 2-4 hours was noted without any clinical adverse effects. In the studied group of pigs, oral clonazepam at 0.5 mg/kg had a similar  $T_{\text{max}}$  and volume of distribution, but a shorter terminal half-life compared to human data. Observational evaluation of clonazepam as an oral tranquilizer in pigs is promising and warrants further investigation before clinical use.

### An Interdisciplinary approach to assessing freshwater mussel health and mortality in the Clinch River

Jeronimo Silva, Dr. Michelle Dennis, Justin Wolbert, Gerald Dinkins, Rebecca Hardman, and Dr. Augustin Engman

Freshwater mussels are among the most imperiled animal taxa globally and regionally, with around 70% of North American species being classified as endangered or threatened. Population declines across the globe have been associated with habitat degradation and fragmentation caused by dams and pollution. Mussel die-offs have also played a role in population declines. Since 2016, mussel die-offs have been noted in the Clinch River, a freshwater biodiversity hotspot flowing across Southwest Virginia into East Tennessee. Although researchers have investigated these events, no study has identified a clear causative factor or mechanism. Our study aims to determine likely causes of die-offs in the Clinch River with a two year long in-situ experiment that measures seasonal changes in health and mortality of hatchery-reared *Actinonaias pectorosa* maintained in silos at two die-off sites. Although an August - November wild mussel die-off event was observed at both sites in the first year of the experiment, no silo mussels presented signs of disease and few mortalities occurred. From May - December 2021, silo mussels grew on average 10.8mm. We have assessed a total of 82 mussels (57 silo mussels and 25 wild mussels). Data on survival and growth, clinical signs of disease, hemolymph indices, histopathology, and bacterial microbiome in free-living and experimental mussels will be paired with historical population demographic and die-off data. These data, in combination with environmental data (i.e., river flow, temperature), will be used to build models that explore likely causes of die-off events and make predictions considering climate change scenarios.

### Serological response of Mallard ducks (*Anas platyrhynchos*) following an experimental infection of *Toxoplasma gondii* tachyzoites

Nicole Szafranski, Wei Wang, Dr. Chunlei Su, and Dr. Rick Gerhold

Waterfowl are unique epidemiological subjects for a variety of disease transmission pathways, including for many zoonotic pathogens, such as the protozoan parasite *Toxoplasma gondii*. *T. gondii* can infect a variety of avian and mammalian species worldwide, including humans, and cause a wide range of clinical disease. While infections have been historically recorded in waterfowl species, little work has been done to understand the immune response that occurs within infected birds. In nature, transmission to birds occurs via the ingestion of oocysts in contaminated food or water or the ingestion of infected meat. As mallard ducks are the most populous and commonly hunted ducks in the United States, they were an ideal species for primary investigation. For this experimental infection, 64 mallard ducks were infected with *T. gondii* tachyzoites, and 10 additional birds acted as an uninfected control group. Infected groups were further divided into four subsets each containing 16 ducks, receiving a high or low dose of tachyzoites from a moderately virulent or strongly virulent known strain of *T. gondii*. Serial serum antibody titers were collected over an infection period of 70 days and analyzed for IgG development via the Modified Agglutination Test (MAT). Overall, there was a seroconversion rate of 93.75% with 60 of the 64 infected birds developing antibody titers ranging from 1:5 up to greater than 1:640. No birds developed any signs of clinical disease throughout the time period. This is a great first step into better understanding the disease development and impact on waterfowl species.

### Cytological investigation of brown pigment lesions in mountain star coral (*Orbicella faveolata*)

Dr. Amy Webb, Dr. Michelle Dennis, and Dr. Anne Becker

Background: Coral reefs have significantly declined over the last several decades, and although disease is a major contributing factor, most diseases affecting coral reefs are poorly understood. A common lesion of several Caribbean corals is dark yellow to brown discoloration of coral tissue followed by central tissue loss, often visually diagnosed as “dark spots disease”. The pathological basis for the pigmentation is presently unknown. Objective: The purpose of this study was to investigate sample preparation methods best for cytological evaluation of coral symbiont (Symbiodiniaceae) morphology with the hypothesis that differences account for the brown pigmentation. Methodology: Samples were collected August 2022 from Half Moon Bay in St. Kitts. All samples were taken from colonies of *Orbicella faveolata* (OFAV) with characteristic lesions. Paired samples, one from affected tissue and one from apparently healthy tissue, were taken from 8 colonies. Samples were collected via airbrush and/or syringe slurry. Slides for cytology were prepared as dry smears or wet mounts. Romanowsky type stain or new methylene blue (NMB) stain was applied. Samples were evaluated on 100x objective with oil. Results: A modified wet mount prepared from slurry samples and stained with NMB yielded the best morphological detail of Symbiodiniaceae. Preliminary data indicate no major cytological difference between Symbiodiniaceae of affected versus healthy tissue which would explain the discolored lesion.

### Development of a F18 labeled peptide p5+14 for PET imaging of peripheral amyloidosis

Eric Webster, Dr. Jon Wall, and Dr. Steve Kennel

Purpose: Systemic amyloidosis is a rare protein misfolding disorder leading to progressive organ failure. Peptide p5+14 has been radiolabeled with I-124 and fully characterized for injection into patients to assess safety and systemic amyloid burden. While imaging of peptide p5 +14 with 124I is promising, the use of [18F], the most abundantly distributed PET isotope, could improve access to the labeled product. Methods: Conventional [18F] labeling processes include harsh reaction conditions that are not usually appropriate for labeling peptides. Recent advances in alternative methods for radiolabeling peptides with [18F] have been developed. The use of a Si-F bond on a moiety coupled to the N terminus has been recently used to label small peptides using an isotopic exchange strategy. We have used this strategy of labeling for peptide p5+14 with [18F]. Results: The non-radioactive starting material (TC-16) was synthesized and lyophilized by an external contractor. A synthesis was developed by following published work using isotopic exchange as the strategy for radiolabeling. Radiolabeling efficiency was measured using radio HPLC. Results: Results for the initial experiments for radiolabeling and purification of the F18 labeled peptide were mixed. Actual radiolabeling of peptide p5+14 was observed on 50% of attempts. In most of these attempts (4 of 5), the yield observed was greater than 50% and radiochemical purity was greater than 80%. Five other attempts produced no radio-labelled peptide product. The details of the methods used that yielded the variable results are being revisited.



### Audioarthrology: A potentially useful tool for assessing joint disease

Molly Werder, Dr. Darryl Millis, Dr. James Lewis, Dr. Marti Drum, and Emily Sutherland

Studies of humans have evaluated the acoustics produced by the knee and the temporomandibular joints, but similar technology has yet to be evaluated in veterinary medicine. The use of a stethoscope to hear and visualize the acoustics associated with joint motion and joint disease has potential to be useful as a diagnostic tool for veterinarians. The application of a stethoscope to characterize joint pathology may decrease the cost of diagnostics for joint injury and disease and may be a minimally invasive tool for diagnosis and monitoring of joint disease. A digital stethoscope (3M™ Littman® CORE stethoscope, Eko Device Inc, Oakland, CA) was used to record sounds from 50 coxofemoral joints during range of motion. The patients selected from the orthopedic service at the University of Tennessee Veterinary Medical Center were assessed for orthopedic disease then imaging was performed to confirm the disease state of the joint. Sounds were extracted from the joint recordings and analyzed in both the time and frequency-domains. Analysis included the decay time of popping sounds and the magnitude and modulation spectra of grinding sounds. It was observed that healthy joints tend to exhibit modulations dispersed over a broader frequency range than dysplastic joints. Based on these results, further analysis into the acoustics of joint sounds for the classification and description of joint disease is warranted.

### Preliminary study of antebrachial circumference measurement and association with muscle mass in dogs

Dr. Sang Chul Woo and Dr. Darryl Millis

The purpose of this study was to evaluate the relationship between antebrachial circumference (AC) and antebrachial muscle mass (AM) in dogs. We hypothesized that there would be a positive correlation between AC and AM. Six canine cadavers were used to measure both forelimbs. Antebrachial Length (AL) was measured from proximal olecranon to lateral styloid process, and AC was measured perpendicularly to the long axis of the limb at 20% of AL distal to the proximal olecranon using a spring tension-measuring tape. The muscles of the antebrachium were dissected, and the mass was determined. The difference between observers on left and right AC measurements was analyzed using one-way repeated measures. Mean AM increased as AC increased, and a positive linear trendline was established ( $r^2 = 0.99$ ). A statistical difference ( $p < 0.01$ ) between mean AC of each dog was identified, but there was no statistical difference ( $p = 0.148$ ) between the AC of left and right forearm of the same dog. The difference of the mean AC between two observers was 0.06 cm on the left and 0.26 cm on the right. There was no significant difference between the observers on either left or right seen by the high  $p$  value, which indicated high interobserver reliability. In conclusion, our study supported that antebrachial circumference has a strongly positive correlation to muscle mass. This study established a repeatable method using common anatomical landmarks to measure muscle mass with reliability and is a stepping stone to measuring muscle atrophy.